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Short Communication

Induction of lung lesions by bronchial administration using bronchoscope technique in mice

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Abstract: This study aimed to establish an exposure method that can induce homogeneous lesions with minimal inter-individual variability. The distribution of lesions induced by bleomycin (BLM) administration was also analyzed. C57BL mice were intrabronchially administered 20 µL of BLM (3 mg/mL) using a bronchoscope in the left or right bronchus. The mice were sacrificed 14 days after administration, and their lungs were evaluated histopathologically. BLM-induced inflammatory lesions were widely observed in the lungs. In the left bronchus-treated group, lesions were uniformly observed throughout the lobe, and no individual differences were noted. Meanwhile, in the right bronchus-treated group, individual differences in the distribution of the pulmonary lesions were observed. The distribution of lesions differed among the four lobes of the right lung owing to their anatomical features. Administration into the left bronchus is recommended for highly homogeneous lung exposure and for establishing models that contribute to highly accurate toxicity and efficacy evaluations. (DOI: 10.1293/tox.2023-0123; J Toxicol Pathol 2024; 37: 93–97)

Key words: bronchi, bleomycin, bronchoscopes, mice, inflammation

Intratracheal administration of compounds has been widely used to establish models of lung injury and to assess the risk of lung toxicity[1–6]. In these studies, lung lesions induced by the compounds were observed randomly in both the left and right lungs across various lobes owing to bronchial branching. This outcome complicated the interpretation of group evaluations in toxicity and efficacy assessments, including the analysis of biological samples such as bronchoalveolar lavage fluid and histopathological analysis. To reduce inter-individual variability, a catheter is inserted into the bronchus, mainly the left bronchus[7, 8]. The catheter is inserted through the trachea into the bronchus during the surgical procedure or by controlling the mouse posture.

Recently, the development of narrow endoscopic systems has facilitated viewing the trachea and bronchi of mice, and catheter insertion into the bronchus can be performed accurately and non-invasively[9]. Selective exposure to the left or right bronchi presumably results in different lesion distributions because rodent lungs differ in lobular status from left to right. To establish an exposure method that can induce homogeneous lesions with little inter-individual variability, we analyzed the distribution of lesions induced by selective compound administration in the left or right bronchi. These results have the potential to contribute to highly accurate toxicity and efficacy evaluation in animal models.

In this study, we selected bleomycin (BLM) exposure in C57BL mice as the test system[10–12]. Male C57BL/6JJcl mice were purchased from CLEA Japan Inc. (Tokyo, Japan) and subjected to experiments at 7 or 8 weeks of age. The mice were housed in cages in an animal room maintained at a temperature of 23 ± 2°C and a humidity of 50% ± 10%, with 9 to 15 air changes per hour and a 12-hour light and 12-hour dark cycle; the mice were fed pelleted chow (CA-1; CLEA Japan Inc.) and tap water *ad libitum*.

Lungs of three 7-week-old mice (body weight: 21.3–22.3 g) were scanned using X-ray micro-computed tomography (CT), CosmoScan FX (TJ00046145-1, Rigaku, Tokyo, Japan). The mice were anesthetized with isoflurane, and the state of anesthesia during scanning was maintained with sevoflurane. Scanning parameters included 90 kV X-ray voltage, 88 µA X-ray current, 25 mm × 25 mm × 25 mm field of view, 50 × 50 × 50 µm spatial resolution, and exposure time of 4 min. After scanning, the mice were euthanized by isoflurane overdose. The imaging data were thresholded based on the signal intensity, and the trachea

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and bronchus were labeled using Analyzed Normalization Tools (http://stnava.github.io/ANTs/) and ITK-SNAP version 3.8.0 (University of Pennsylvania, State College, PA, USA). Branch diameters and angles were calculated using Osiris (University of Geneva, Geneva, Switzerland) and Amira 3D (Thermo Scientific, Tokyo, Japan).

Eight-week-old mice were divided into four groups as follows: left bronchus-control group, where saline was injected into the left bronchi of three mice; left bronchus-treated group, where BLM was injected into the left bronchi of 13 mice; right bronchus-control group, where saline was injected into the right bronchi of three mice; and right bronchus-treated group, where BLM was injected into the right bronchi of 20 mice. The dose of BLM was 20 μL/mouse (60 μg/mouse), and the observation period was 14 days; this study design was most suitable for searching for the range of tissue damage caused by inflammation. In a preliminary study, a large variation was observed between individuals in the right bronchus group. Therefore, more mice were assigned to the right bronchus-treated group than the left bronchus-treated group.

BLM (Nippon Kayaku Co. Ltd., Tokyo, Japan) was dissolved in saline and adjusted to a concentration of 3 mg/mL. Mice were anesthetized by mixing anesthesia (medetomidine, 0.2 mg/kg; midazolam, 6 mg/kg; butorphanol tartrate, 10 mg/kg)[13] and administrated subcutaneously at 0.1 mL/10 g body weight. A bronchoscope (AVS narrow endoscope system AE-C1; AVS Co., Ltd., Tokyo, Japan) with a catheter was inserted into the trachea of the mice. After confirming that the bronchoscope with the catheter (20G SURFLO indwelling needle, SR-OT2051C, TERUMO, Tokyo, Japan) reached the bronchus and that the bronchoscope was withdrawn, the catheter was placed in the bronchus, and saline or BLM solution was administered via the catheter using a Hamilton syringe (25 μL Microliter Syringe Model 702N, 80465, Hamilton Company, Reno, NV, USA) and removal needle (22G Small Hub RN Needle 2 in, 7770-02, Hamilton Company) (Fig. 1).

During the study period, clinical signs were observed daily in all mice. All mice were weighed on day 0 (dosing day) and 3, 7, 10, and 14 days post-injection. On day 14, mice were euthanized by exsanguination under isoflurane anesthesia. Mice with the following signs were euthanized using humane endpoints: 1) decrease in weight by >30% compared to that at the time of BLM administration, and 2) significant deterioration in general condition, such as tachypnea, emaciation, and coarse hair.

At necropsy, the lungs of each mouse were infused via the trachea with 10% neutral-buffered formalin (10% NBF) until the thoracic cavity was filled under thoracotomy. After infusion, the lungs were removed and immersed in 10% NBF. The lungs were embedded in paraffin such that all lobes appeared on one surface. Thin sections were prepared and stained with hematoxylin and eosin. To analyze the exposure area of the solution administered to the lungs, we defined a series of inflammatory changes known to be induced by BLM as lesions, and the extent of the lesion (the ratio of the lesion to normal tissue) was scored according to histopathological observations (scores 0–4: 0, no lesion; 1, less than 1/4; 2, 1/4–2/4; 3, 2/4–3/4, and 4, more than 3/4) within each lung lobe of each mouse. Scores were obtained by two certified toxicological pathologists.

Table 1. Branch Measurements and Angles of Bronchi

Diameters (mm)		Angles (°)	
LMB	0.57 ± 0.07	LMB-RMB	54 ± 4
RMB	0.78 ± 0.02	RMB-CrB	135 ± 8
CrB	0.40 ± 0.06	RMB-MiB	40 ± 3
MiB	0.45 ± 0.11	RMB-AcB	37 ± 2
AcB	0.35 ± 0.04		
CaB	0.72 ± 0.08		

Each value represents the mean ± SD. LMB: left main bronchus; RMB: right main bronchus; CrB: cranial lobe bronchus; MiB: middle lobe bronchus; AcB: accessory lobe bronchus; CaB: caudal lobe bronchus.

The bronchial branching is illustrated in Fig. 2A and 2B. The left main bronchus was narrower than the right. The right main bronchus initially branched into the cranial lobe bronchus, followed by the accessory lobe bronchus (AcB), and middle lobe bronchus at approximately the same location. After branching to the AcB, the remaining bronchi were distributed in the caudal lobe bronchi. This branching pattern is similar to that in the previous report[14, 15]. The diameters and angles of the lobar bronchi are listed in Table 1. The diameter of each bronchus in 7-week-old mice was smaller than that observed in a previous study using 20-week-old mice[14, 15]. However, the branching angles of the bronchi are similar. These results suggest that, during the growth process, the diameter expands; however, the angle does not change.

Similar to the CT analysis, AVS narrow endoscopic observation of the left and right main bronchi revealed that the diameters of the left main bronchus were smaller than those of the right main bronchus (Fig. 2C). Although differences in the diameters were noted, the insertion of a catheter into each of the left or right main bronchi and administration of the compounds were possible using an AVS narrow endoscope.

Body weights of the left and right bronchus control groups continued to increase until the end of the observation period (Fig. 3). The left bronchus-treated group demonstrated little inter-individual variability in body weight. At 3 or 7 days after administration, body weight transiently decreased and continued to increase until the end of the observation period (Fig. 3A). The right bronchus-treated group demonstrated greater inter-individual variability. Body weight decreased 3 or 7 days after administration and remained low (Fig. 3B). The moribund cases with continuous weight loss were observed in 1/13 (8%) mice in the left bronchus-treated group and 10/18 (56%) mice in the right bronchus-treated group. Moribund mice exhibited tachypnea, decreased activity, emaciation, and coarse hair. In the planned autopsy cases, tachypnea, decreased activity, emaciation, and coarse

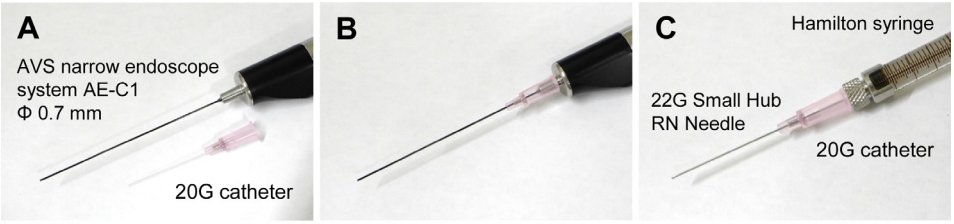

Fig. 1. Method of use for intrabronchial administration. A: Bronchoscope and 20G catheter. B: The bronchoscope was placed in the 20G catheter. C: A Hamilton syringe with a 22G Small Hub RN needle placed in the 20G catheter.

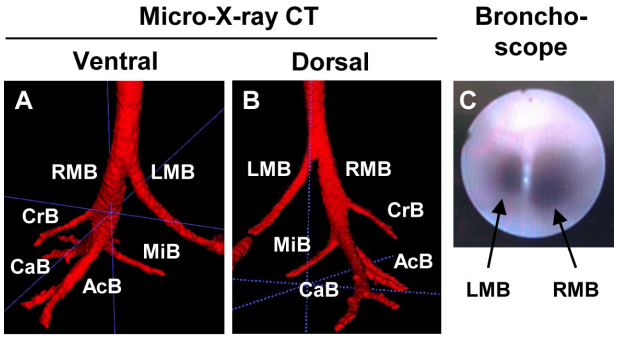

Fig. 2. Bronchial status (nature) of mice. A, B: Bronchial branching analysis using X-ray micro-computed tomography. C: Image obtained by the bronchoscopic probe. LMB: left main bronchus; RMB: right main bronchus; CrB: cranial lobe bronchus; MiB: middle lobe bronchus; AcB: accessory lobe bronchus; CaB: caudal lobe bronchus.

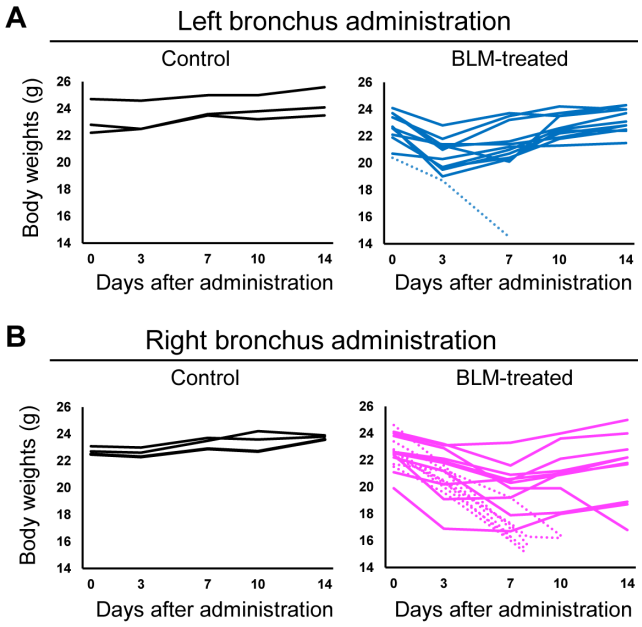

Fig. 3. Body weight change of mice intrabronchially administered with bleomycin (BLM). Mice were administered with saline (control) or BLM into the left (A) and right (B) bronchi. Lines represent the change of each mouse. Solid lines indicate surviving mice, and dotted lines indicate dead/euthanized mice, respectively.

hair were observed in a few mice.

No abnormal pathological changes were observed in the left and right bronchi in the control groups (Fig. 4A). Lesions were observed from the bronchi to the alveoli in BLM-treated mice (Fig. 4B–4D). In the alveoli, infiltration of inflammatory cells composed mainly of mononuclear cells, hyperplastic type II epithelium, and proliferation of fibroblast-like interstitial cells were observed (Fig. 4C). Bronchiolization was also observed in the alveolar region (Fig. 4D). Epithelial hyperplasia was observed in the bronchi and bronchioles. These changes are consistent with previously reported BLM-induced lung lesions[1, 16].

Lung lesions were widely observed in the left bronchus-treated group of the planned autopsied mice. Ten of the twelve cases were grade 4, and the spread of the lesions was relatively uniform (Fig. 5, Supplementary Fig. 1). In addition, slight lesions (grade 1) in the right lung were detected in 5 of 12 cases. In the right bronchus-treated group, a marked difference in the number of lobes and grades of lesions was observed between each lobe and individual mice. In addition, 8 of 10 cases had grade 1 or 2 lesions in their left lungs.

In moribund mice, lesions were observed in all lobes of the right and left lungs (Fig. 6). These subjects demonstrated persistent weight loss and a worsening general condition, which may have been caused by the spread of inflammation in both the left and right lungs. The factors contributing to contralateral exposure are unclear; however, as the catheter is placed near the bifurcation of the left and right bronchi and during administration, the dosing solution may accumulate near the bifurcation and flow to the contralateral side. The right bronchus-treated group had many cases of contralateral lung exposure, which was possibly related to the anatomical characteristics of the bronchus branching into each lobe. Because the diameter of the right bronchus was large, the dosing solution tended to remain in the anterior part of the bronchus and flowed to the contralateral side.

A summary (group average of inflammation grades) of the histopathological analysis of the planned autopsied mice is presented in Fig. 7. Administration to the left bronchus induced uniform lesions in the left lung with less influx into the right lung. Based on these results, administration to the left bronchus is recommended for toxicity evaluation and

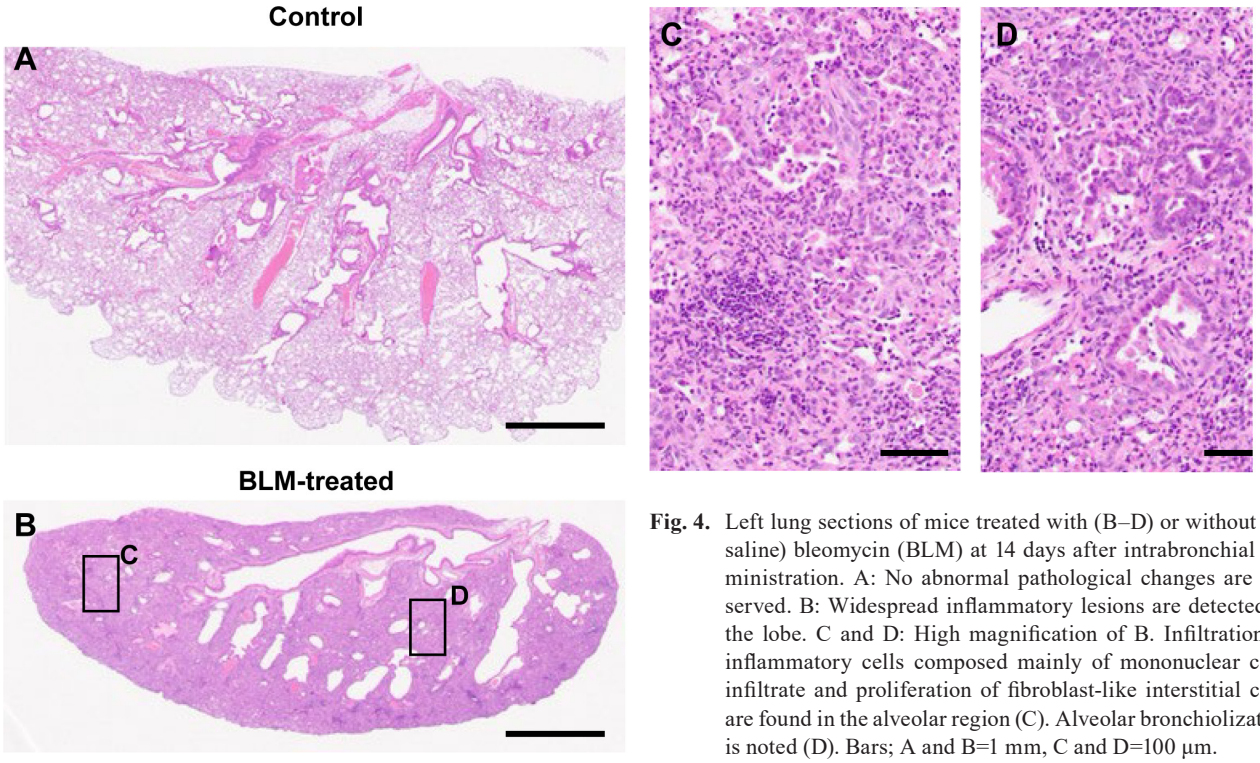

Fig. 4. Left lung sections of mice treated with (B–D) or without (A: saline) bleomycin (BLM) at 14 days after intrabronchial administration. A: No abnormal pathological changes are observed. B: Widespread inflammatory lesions are detected in the lobe. C and D: High magnification of B. Infiltration of inflammatory cells composed mainly of mononuclear cells infiltrate and proliferation of fibroblast-like interstitial cells are found in the alveolar region (C). Alveolar bronchiolization is noted (D). Bars; A and B=1 mm, C and D=100 μm.

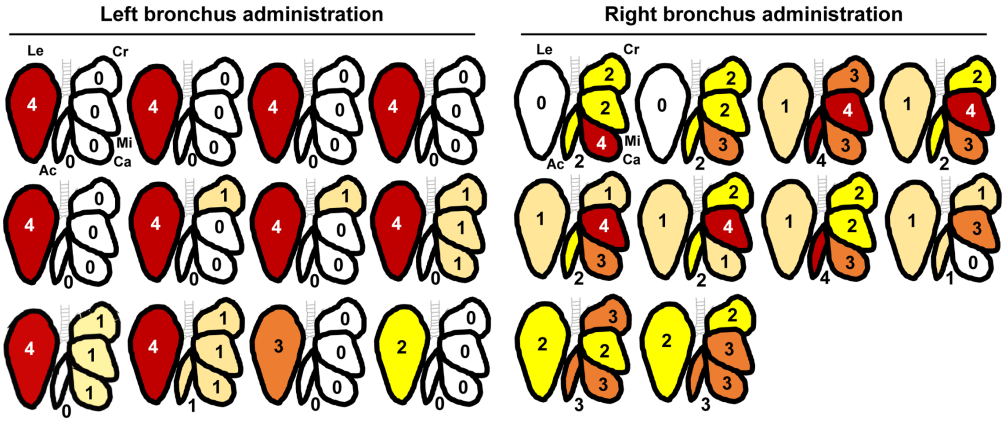

Fig. 5. Distribution of inflammatory lesions in planned autopsy mice administered with bleomycin (BLM) in the left and right bronchi. The lung schema indicates the inflammation grade of each lung lobe. Color and number indicate the inflammation grade of the lung lobe (scores 4=dark brown, 3=orange, 2=yellow, 1=light beige, and 0=white). Le: left lobe; Cr: cranial lobe; Mi: middle lobe; Ca: caudal lobe; Ac: accessory lobe.

creation of animal models of compound exposure. Bronchoscopy is a powerful tool to achieve uniform exposure during selective bronchial administration.

In this study, the contralateral influx was observed in a few cases when 20 μL/mouse was administered to the left bronchus. The frequency of influx to the right side could be reduced by decreasing the volume of the solution administered or by devising solution conditions, and that the exposure could be more specific to the left lung. When the selectivity for the left lung lobe is increased, conducting an experimental pathological analysis of the same individual may be possible, with the left lobe as the exposed lung and the right lung as the control.

Disclosure of Potential Conflicts of Interest: The authors have no competing interests to declare.

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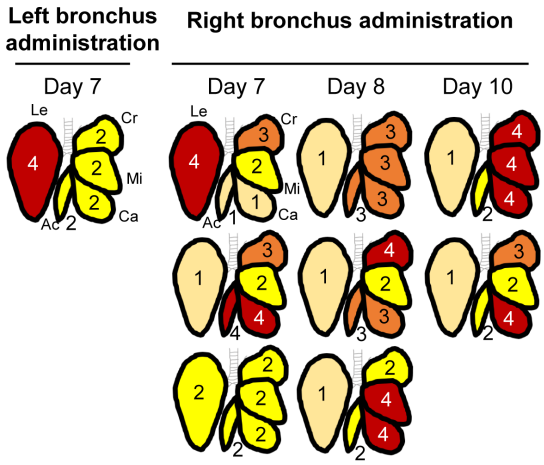

Fig. 6. Distribution of inflammatory lesions in moribund mice administered with bleomycin (BLM) in the left and right bronchi. The lung schema indicates the inflammation grade of each lung lobe. Color and number indicate the inflammation grade of the lung lobe (scores 4=dark brown, 3=orange, 2=yellow, and 1=light beige). Le: left lobe; Cr: cranial lobe; Mi: middle lobe; Ca: caudal lobe; Ac: accessory lobe.

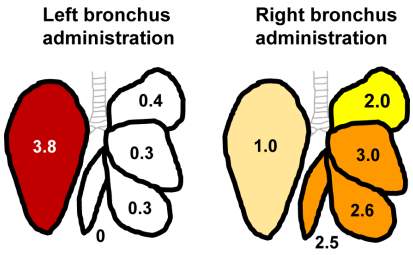

Fig. 7. Summary of inflammation grade score in planned autopsy mice intrabronchially administered with bleomycin. The number indicates the average inflammation grade score.

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